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L4: Entry 7 of 21 File: USPT Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6083685 A
TITLE: Systematic extraction, amplification and detection of retroviral sequences, and oligonucleotides for use therein

Priority Application Year (1):
1995

Brief Summary Text (7):
The most conserved region of retroviral genomes is the primer binding site (PBS). Specific PBS-derived oligonucleotides, or part of tRNA itself, have been used as hybridisation probes or primers in a primer extension reaction (Kroger et al (1987) J. Virol. 61:2071-5).

Detailed Description Text (6):
In a third aspect of the invention, unambiguously defined PBS-derived primers (SEQ ID No 25-48) are used for the systematic amplification of the retroviral sequences in ordered combinations with a limited number of antisense primers derived from other conserved parts of retroviral genome (protease, reverse transcriptase; see FIG. 1). In this case, however, PBS primers are complementary (not identical) to the last 18 nucleotides of

Detailed Description Text (8):
Primers of the second set (antisense primers) are complementary to conserved domains of reverse transcriptase (domains 1-7) and protease. They are degenerate, so that a few primers from a particular domain (in fact only one in domain 5) can be used for amplification of all or most of known retroviral sequences.

Detailed Description Text (9):
The sense PBS primers are identical to PBS which are complementary to the last 18 nucleotides of tRNAs described as primers for exogenous and endogenous human retroviruses, but also those derived from last 18 nucleotides of human tRNAs not yet described to prime retroviruses. These sense primers (see also SEQ ID Nos. 25-48) are reverse complements of the above anti-PBS capture oligonucleotides and are shown in the same order:

Detailed Description Text (10):
Examples of antisense primers are shown below. A limited number of primers derived from particular conserved regions can amplify existing known exo- and endogenous retroviruses. Other conserved region(s) of retroviral genome can be used to design similar sets of primers. The conserved domains of RT are numbered according to Xiong et al, supra.

Detailed Description Text (11):
Primers described in the third aspect of the invention or other primers derived from conserved regions of the RT or protease genes can be double labelled with reporter and quencher fluorophores using Taqman technology (Perkin-Elmer). These labelled probes have to be positioned internally in respect of PCR primers. When added to the reaction, the signal is created only when the target sequence is amplified. This can not only confirm that using a particular combination of primers yields the amplified product without analysing it on the gel, but using group-specific antisense primers labelled with various reporter fluorophores (differing in emitting wavelengths) can directly classify amplified sequence.

Detailed Description Text (12):
Specific antisense primers (see also SEQ ID Nos. 49-57) are:

Detailed Description Text (15):
If used at the same time for classification, the primers from domain 3 PT (SEQ ID Nos. 49-53) can be used as probes if primers from domain 4 (SEQ ID Nos. 54-56) or primer from domain 5 (SEQ ID No. 57) are used as antisense PCR primers. Similarly, primers from domain 4 (SEQ ID Nos. 54-56) can be used as probes if primer from domain 5 (SEQ ID No. 57) is used as antisense PCR primer etc.

Detailed Description Text (21):
The use of biotinylated anti-PBS primers as tools for rapid detection and transcription pattern analysis of retroviral transcripts is based on an observation that, in a vast majority of retroviral genomes, the first splice donor site is located downstream of PBS (see FIG. 1), and PBS becomes part of the leader sequence present in the majority if not all RNA transcripts. As mentioned, it can be a very useful approach for comparative qualitative and quantitative studies. Preliminary experiments showed an expected hybridisation pattern using control Northern blots prepared on RNA extracted from cells infected with known viruses. Hybridisation of .sup.32 P-labelled oligo corresponding to 3'-end of tRNA Lysine 3 known to prime HIV 1 and 2 (SEQ ID No. 2) to the total and poly (A)+ RNA from uninfected and HIV1-infected CEM cells showed some non-specific hybridisation on total RNA from uninfected cells; in mRNA fractions, only poly(A)+ from infected cells showed a hybridisation signal corresponding to several mRNA species.

Detailed Description Text (23):
The technique has been verified using DNA and RNA from cells infected with HIV and HTLV viruses. The reaction conditions have been optimised in respect of non-degenerate:degenerate primer ratio (optimal ratio 1:6), annealing temperature etc. The specific products have been confirmed by hybridisation to virus-specific labelled probes prepared from the corresponding cloned viral genomes. The size of expected PCR products for various retrovirus is between 2.0 and 3.5 kilobase (including gag, protease and major part of RT gene). The amplified HTLV I and HIV 1 PCR products were close to 2.1 and 2.3 kb when using antisense primers from domains 3 (SEQ ID No. 50 and 53, respectively) and 5 (SEQ ID No. 57) of the reverse transcriptase, and corresponding PBS-derived primers (SEQ ID Nos. 25 and 25, respectively).

Detailed Description Text (24):
If the aim is to look for retroviral sequences (e.g. HERV sequences) which are transcriptionally active, then the template should be RNA as explained earlier. The conditions have been established (see Examples, 1.3.) for the successful RT PCR using captured RNA and primers from two sets (sense; SEQ ID Nos. 25-45; and antisense, SEQ ID Nos. 49-57).

Detailed Description Text (27):
1.1. Retroviral RNA Capture When plasma or serum samples are analysed the RNA can be directly captured with biotinylated anti-PBS oligonucleotides (SEQ ID Nos. 1-24) and streptavidin coated paramagnetic particles (SPMP), starting with 1.1.3. The analysis of intracellular RNA works well when a total RNA is first extracted (1.1.1.) followed by the removal of small RNA molecules by LiCl precipitation (1.1.2.). The LiCl precipitation and the capture procedure should be preceded by a short denaturation to remove any annealed natural primer tRNA molecules which could interfere with a capture.

Detailed Description Text (54):
To confirm the specificity of control reactions, Southern blotting and hybridisation to specific probes were carried out using standard techniques. The PCR products of supposedly unknown origin were cloned, sequenced and analysed. A semi-nested PCR using an internal antisense primer was sometimes used to confirm retroviral origin of the amplified product.

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L4: Entry 15 of 21 File: USPT Oct 27, 1998

DOCUMENT-IDENTIFIER: US 5827935 A
TITLE: Chimeric tRNA.sup.lys ribozyme molecules

Abstract Text (1):
The invention provides novel chimeric tRNA.sup.LYS -ribozyme molecules that compete effectively with tRNA.sup.LYS for HIV-1 reverse transcriptase binding sites. The chimeric human tRNA.sup.LYS ribozymes inhibit reverse HIV transcription by delivering inhibitors such as ribozymes of HIV-1 reverse transcriptase directly to the virion particle and render it non-functional. The chimeric molecules of the invention thus serve as highly specific non-toxic therapeutic agents. These chimeric molecules also reveal a novel, site specific RNA cleaving activity of HIV-1.

Priority Application Year (1):
1992

Brief Summary Text (2):
This invention relates to chimeric tRNA.sup.LYS ribozyme molecules which compete effectively with tRNA.sup.LYS for binding to HIV-1 reverse transcriptase. These chimeric molecules provide a mechanism for delivering inhibitors of HIV-1 transcriptase to the virion particle itself.

Brief Summary Text (4):
It has been demonstrated that the entire tRNA.sup.LYS molecule as well as various segments of the tRNA per se are capable specifically of interacting with HIV-1 transcriptase. See Barat, et al. EMBO Journal 8:3279-3285 (1989); Khan, et al. J. Bio. Chem 267:6689-6695 (1992); Weiss, et al., Gene 111:183-197 (1992). Ben-Artzi, Proc. Natl. Acad. Soc. USA 89:927-931 (1992) reports an RNAse cleavage activity associated with HIV-1 reverse transcriptase. This activity is shown to cleave only HIV-1 RNA, not the primer.

Brief Summary Text (5):
Prior to this invention there has been no report of chimeric tRNA.sup.LYS -ribozyme molecules.

Brief Summary Text (7):
This invention provides novel chimeric tRNA.sup.LYS ribozyme molecules that compete effectively with tRNA.sup.LYS for HIV-1 reverse transcriptase binding sites. The chimeric human tRNA.sup.LYS ribozymes inhibit reverse HIV transcription by delivering inhibitors such as ribozymes of HIV-1 reverse transcriptase directly to the virion particle and render it non-functional. The chimeric molecules of this invention thus serve as highly specific non-toxic therapeutic agents.

Drawing Description Text (2):
FIG. 1 shows the structure of one chimeric ribozyme. This tRNA.sup.LYS -ribozyme construct has been cloned into a Blue Script transcription vector using SacII and XhoI restriction sites. Following linearization at the SacII site the chimeric RNA can be transcribed in vitro using bacteriphage T-7 RNA polymerase. There is also a Mae I restriction site in between the tRNA and ribozyme moieties, allowing the tRNA to be transcribed independently of the ribozyme.

Drawing Description Text (3):
FIG. 2. This gel shift experiment shows binding of the chimeric tRNA.sup.LYS ribozyme

to HIV-1 reveres transcriptase. The eight lanes of the gel from left to right are:

Drawing Description Text (5):
2. tRNA.sup.LYS in vitro transcript which has extra bases at both the 5' and 3' ends. The extra 5' bases are from the Blue Script poly linker between the T-7 promoter and the XhoI site. There are six extra nucleotides at the 3' derived from the nucleotides after the CCA of the tRNA to the Mae I site which separates the tRNA from the ribozyme.

Drawing Description Text (6):
3. tRNA.sup.LYS -ribozyme in vitro transcript which has the same extra 5' bases as tRNA.sup.LYS, but terminates at SacII site at the end of the ribozyme moiety.

Drawing Description Text (8):
5. tRNA.sup.LYS -ribozyme transcript incubated with HIV-1 reverse transcriptase.

Drawing Description Text (10):
7. tRNA.sup.LYS -ribozyme incubated with AMV reverse transcriptase.

Drawing Description Text (11):
8. tRNA.sup.LYS with competing, non-radioactively labelled tRNA.sup.LYS -ribozyme incubated with HIV-1 reverse transcriptase.

Drawing Description Text (12):
This FIG. 2 shows that the chimeric tRNA.sup.LYS -ribozyme specifically binds to HIV-1 reverse transcriptase by a shift in radioactivity when HIV-1 reverse transcriptase is present. Cold tRNA.sup.LYS -ribozyme competes with tRNA.sup.LYS for binding to HIV-1 reverse transcriptase as indicated by the reduced radioactive shift in lane 8.

Drawing Description Text (13):
FIG. 3. This experiment demonstrates cleavage of a 162 nucleotide, radioactively labelled HIV-1 RNA containing the primer binding site plus sequences upstream of this and including the AUC cleavage signal for the ribozyme. The cleavage products are 101 and 61 bases. The extent of cleavage increases with increasing temperature.

Detailed Description Text (2):
Genetic fusions consisting of the entire mature coding sequence or 18 bases of the 3' end of human tRNA.sup.LYS have been fused to hammerhead ribozyme containing RNAs with base pairing capabilities to the HIV-1 sequences immediately 5' or upstream of the primer binding site. The 3' terminal 18 nucleotides of the tRNA.sup.LYS are complementary to the primer binding site.

Detailed Description Text (3):
These chimeric molecules have been tested in cell free assays for their ability to bind to HIV-1 reverse transcriptase and their inhibitory activity on HIV-1 reverse transcriptase polymerization activity. The ribozyme moiety targets the cleavage of HIV-1 viral RNA at a known hammerhead cleavage site immediately upstream of the primer binding site for initiation of reverse transcription in the HIV-1 viral RNA. The site chosen for initial study, and reported here is an AUC in which cleavage is immediately after the C. This site is absolutely conserved in all HIV-1 isolates sequenced to date. The chimeric RNAs, which are specifically bound by HIV-1 reverse transcriptase should be carried into newly formed HIV-1 virions during viral assembly. The chimeric primers effectively block HIV-1 reverse transcription, making them a novel, highly target specific, and unique anti-HIV-1 therapeutic agent. In addition, the tRNA.sup.LYS portion contains within its mature coding sequence the elements required for transcription by human RNA polymerase III, thereby making it feasible to insert the gene, rather than the RNA into human cells.

Detailed Description Text (4):
Studies of the binding of the chimeric molecules to HIV-1 reverse transcriptase, revealed that the complex of chimeric tRNA.sup.LYS -ribozyme, or 18 3' nucleotides of tRNA.sup.LYS -ribozyme, or tRNA.sup.LYS with an extra 6 nucleotides appended to the 3' end, when base paired to the primer binding site signal of HIV 1 RNA, serves as a substrate for a novel ribonuclease activity associated with HIV-1 reverse transcriptase. This activity results in cleavage of the primer at a site very close to

the 3' end of the tRNA.sup.LYS molecule, CCA-3'. This activity is of unknown function in the viral replication cycle, but may play an important role in the use of chimeric RNAs by freeing the ribozyme moiety from the tRNA moiety such that it can cleave one or both of the viral RNAs encapsidated in the HIV-1 virion.

Detailed Description Text (6):
The idea of chimeric tRNA.sup.LYS -ribozyme molecules which effectively compete with tRNA.sup.LYS for binding to HIV-1 reverse transcriptase is novel. It provides a possible mechanism for specifically delivering inhibitors of HIV 1 reverse transcriptase to the virion particle itself. Such inhibitory agents will render these viral particles non-functional, and thus serve as highly specific, non-toxic therapeutic agents.

Detailed Description Text (7):
It has been demonstrated that the entire tRNA.sup.LYS molecule, as well as various segments of the tRNA itself are capable of specifically interacting with HIV-1 reverse transcriptase. No one has shown that chimeric molecules such as the the ones described could specifically bind to HIV-1 reverse transcriptase. No other work has described that such molecules are inhibitory to HIV-1 reverse transcriptase polymerase activity. There is one published report of an RNAse cleavage activity associated with HIV-1 reverse transcriptase. This activity was only shown to cleave HIV-1 RNA, not the primer. This activity cleaves twice in the primer binding site, and only substrates paired with tRNA.sup.LYS.

Detailed Description Text (8):
The RNA attached to the 3' end of the tRNA.sup.LYS need not be a ribozyme, but any extra RNA which can base pair with the HIV-1 target upstream of the primer biding site. If a ribozyme is joined to the tRNA, other cleavage sites such as CUC, or CUA which are on the HIV-1 sequence just to the 3' side (downstream) of the AUC site can be targeted. It is not necessary to make an entire tRNA.sup.LYS -ribozyme fusion because it is now known that the last 18 nucleotides of tRNA.sup.LYS fused to the ribozyme are also bound by HIV-1 reverse transcriptase. Genetic variants of tRNA.sup.LYS which compete better than tRNA.sup.LYS for binding to HIV-1 transcriptase are included in the invention.

Detailed Description Text (9):
The ribozyme fusions to tRNA.sup.LYS allow specific targeting of the ribozyme to the HIV-1 virion. Since all retroviruses use cellular tRNAs for priming, this invention provides a general strategy for inhibiting other retroviruses as well. Existing ribozyme technology makes use of specific base pairing between ribozyme and target, but this is accomplished by diffusion of the ribozyme until it finds a target RNA. This invention uses well known retroviral packaging pathways to specifically carry the ribozyme into the virion, and get it bound to the correct site on the viral RNA for cleavage.

CLAIMS:

1. A chimeric tRNA.sup.LYS -ribozyme.

2. A chimeric human tRNA.sup.LYS -ribozyme.

3. A construct comprising a ribozyme fused to a coding sequence of the 3' end of tRNA.sup.LYS, said coding sequence being capable of base pairing with an HIV-1 viral RNA sequence.

5. The construct according to claim 3 wherein said ribozyme is a hammerhead ribozyme.

6. The construct according to claim 4 wherein said ribozyme is a hammerhead ribozyme.

7. A complex as depicted by FIG. 1, wherein SEQ ID NO. 1 specifies the HIV-1 mRNA of FIG. 1 and SEQ ID NO. 2 specifies the tRNA.sup.LYS -ribozyme construct of FIG. 1.

8. A molecule comprising at least the eighteen bases of the 3' end of tRNA.sup.LYS fused to a ribozyme moiety, said ribozyme moiety having an RNA sequence which base pairs with an HIV-1 viral RNA sequence immediately 5' of the primer binding site for

initiation of reverse transcription in HIV-1 viral RNA, said ribozyme moiety being selected to target a cleavage site of said RNA sequence immediately 5' of the said primer binding site for initiation of reverse transcription in HIV-1 viral RNA.

10. The molecule according to claim 8, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

11. The molecule according to claim 9, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

12. A complex comprising HIV-1 reverse transcriptase bound to a chimeric tRNA.sup.LYS -ribozyme, said chimeric tRNA.sup.LYS ribozyme comprising a tRNA.sup.LYS moiety and a ribozyme moiety, wherein the ribozyme moiety is capable of base pairing with an HIV-1 viral RNA sequence immediately 5' of the primer binding site for initiation of reverse transcription.

14. The complex according to claim 12, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

15. The complex according to claim 13, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

16. A nucleic acid complex comprising HIV-1 viral RNA bound to a chimeric tRNA.sup.LYS -ribozyme, said chimeric tRNA.sup.LYS ribozyme comprising a tRNA.sup.LYS moiety and a ribozyme moiety, wherein said tRNA moiety is bound to a primer binding site for initiation of reverse transcription of the HIV-1 viral RNA.

18. The nucleic acid complex according to claim 16, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

19. The nucleic acid complex according to claim 17, wherein said ribozyme moiety is a hammerhead ribozyme moiety.

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L4: Entry 2 of 21 File: USPT Oct 2, 2001

DOCUMENT-IDENTIFIER: US 6297004 B1
TITLE: Recombinant viruses displaying a nonviral polypeptide on their external surface

Priority Application Year (1):
1993

Priority Application Year (2):
1993

Brief Summary Text (52):
In the case of a retroviral genetic display package, long terminal repeat sequences, a tRNA primer binding site and a polypurine tract should preferably be included to ensure reverse transcription and integration of the encapsidated RNA in an infected target cell. It may also be desirable to include a selectable marker gene in the encapsidated nucleic acid to facilitate recovery of sequences encoding the displayed polypeptide from infected target cells after a round of selection.

Brief Summary Text (67):
The nucleic acid of the viral particles may be engineered to contain a sequence for transcription of an RNA product, or for expression of a protein, by the infected cells. A whole range of proteins, peptides, antisense RNA transcripts and ribozyme sequences could be encoded within the virion for therapeutic effect, as illustrated by:

Brief Summary Text (69):
b) Intracellular immunisation, for example targeted in vivo delivery (to CD4 expressing cells) of genes encoding proteins, antisense transcripts or ribozymes which interrupt or abort HIV life cycle following virus entry.

Brief Summary Text (71):
d) Cancer therapy. Delivery of genes encoding proteins which destroy the target cell (for example, a ribosomal toxin), indirectly stimulate destruction of target cell by natural effector cells (for example, strong antigens to stimulate immune system) or convert a precursor substance to a toxic substance which destroys the target cell (for example, a prodrug-activating enzyme). Encoded proteins could also destroy bystander tumour cells (for example with secreted antitumour antibody-ribosomal toxin fusion protein), indirectly stimulate destruction of bystander tumour cells (for example cytokines to stimulate immune system or procoagulant proteins causing local vascular occlusion) or convert a precursor substance to a toxic substance which destroys bystander tumour cells (e.g. enzyme which activates prodrug to diffusible drug). Also, delivery of genes encoding antisense transcripts or ribozymes which interfere with expression of cellular genes critical for tumour persistence (for example against aberrant myc transcripts in Burkitts lymphoma or against bcr-abl transcripts in chronic myeloid leukaemia).

Brief Summary Text (133):
Replication-defective viruses displaying antibodies and other nonviral peptides, polypeptides or glycopolypeptides and encapsidating genes encoding therapeutic products (eg proteins, ribozymes, antisense RNA) could be used to achieve efficient and selective delivery and expression of the encapsidated genes to target cells (which may be stem cells, differentiated cells or transformed cells of any tissue of origin), to stimulate the target cell to divide or to enter a specific programme of

differentiation at the time of contact between virus and target cell, or for virus purification on a solid support coated with an antigen which binds to an altered surface component of the virus.

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End of Result Set

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L1: Entry 1 of 1 File: USPT Mar 12, 2002

DOCUMENT-IDENTIFIER: US 6355415 B1
TITLE: Compositions and methods for the use of ribozymes to determine gene function

Brief Summary Text (22):
Without intending to limit the promoter sequence to any particular sequence, in one preferred embodiment, the promoter sequence comprises an adenovirus type 2-associated RNA I gene promoter sequence. In a yet more preferred embodiment, the promoter sequence further comprises a promoter sequence selected from the group consisting of tRNA, CMV, RSV, SV40, PEPCK, MT, SR.alpha., P450 family, GAL7, T.sub.7, T.sub.3, SP6, K11 and heat shock protein promoter sequences. In an alternative preferred embodiment, the promoter sequence comprises a CMV promoter sequence, a T.sub.7 promoter sequence, and a vaRNA I promoter sequence.

Detailed Description Text (21):
Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription [Maniatis, T. et al., Science 236:1237 (1987)]. Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in plant, yeast, insect and mammalian cells and viruses (analogous control elements, i.e., promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. The term "promoter sequence" as used herein refers to a single promoter sequence as well as to a plurality (i.e., one or more) of promoter sequences which are operably linked to each other and to at least one DNA sequence of interest. For example, one of skill in the art knows that it may be desirable to use a double promoter sequence (i.e., a DNA sequence containing two promoter sequences) or a triple promoter sequence (i.e., a DNA sequence containing three promoter sequences) to control expression of a DNA sequence of interest. Double promoters are exemplified, but not limited to, vaRNA I-tRNA, vaRNA I-CMV, vaRNA I-RSV, vaRNA I-SV40, vaRNA I-PEPCK, vaRNA I-MT, vaRNA I-SR.alpha., vaRNA I-P450 family, vaRNA I-GAL7, T.sub.7 -vaRNA I, T.sub.3 -vaRNA, vaRNA I-SP6, vaRNA I-K11, and vaRNA I-heat shock protein double promoters, while triple promoters are exemplified, but not limited to, the CMV-T.sub.7 -vaRNA I triple promoter.

Detailed Description Text (44):
As used herein, "substrate RNA" refers to a ribonucleotide sequence with which a ribozyme is capable of hybridizing and cleaving. Substrate RNA includes, but is not limited to, messenger RNA (mRNA) (including, for example, eukaryotic mRNA and prokaryotic polycistronic mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA), heterogeneous nuclear (also known as pre-messenger) RNA (hnRNA), small nuclear RNA (snRNA), genomic RNA (e.g., in retroviruses), and chemically synthesized ribonucleotide sequences. RNA may be encoded by a complementary DNA template or RNA template.

Detailed Description Text (73):
Ribonuclease P contains a catalytic RNA and a small subunit protein. It was discovered in bacteria and is able to generate a mature 5' end of tRNA by endonucleocatalytic cleavage of precursor transcripts (Guerrier-Takada et al. (1983) Cell 35: 849-857).

Detailed Description Text (106):
The invention is not limited to the vaRNA I gene promoter. Other promoters which are

included within the scope of the invention include, but are not limited to, tRNA promoter, 5S rRNA promoters, histone gene promoters, CMV promoter (located between positions +1 to +596 in vector plasmid pCR3 from Invitrogen), RSV promoter (can be isolated from vector plasmid pRc/RSV from Invitrogen), SV40 promoter (located between positions +3530 to +3192 in vector plasmid pCR3 from Invitrogen), PEPCK promoter, MT promoter, SR.alpha. promoter, P450 family promoters, GAL7 promoter, T.sub.7 promoter having the 23-bp sequence (SEQ ID NO:2) 5'-TAATACGACTCACTATAGGGCGA-3'), T.sub.3 promoter having the 24-bp sequence (SEQ ID NO:3) 5' TTATTAACCCTCACTAAAGGGAAG-3', SP6 promoter having the 23-bp sequence (SEQ ID NO:4) 5'-ATTTAGGTGACACTATAGAATAC-3', and K11 promoter. The T7 promoter, T.sub.3 promoter, SP6 promoter and K.sub.11 promoter have been described in U.S. Pat. No. 5,591,601, the entire contents of which are incorporated by reference.

Detailed Description Text (176):
Plasmid pT.sub.7 T.sub.7 was used to provide a T.sub.7 polymerase gene controlled by T.sub.7 promoter. pT.sub.7 T.sub.7 was constructed as previously described by Wagner et al., U.S. Pat. No. 5,591,601, the entire contents of which are incorporated by reference. Briefly, a fragment of pAR1173 containing a T.sub.7 gene was inserted into pTM-1 vector [Wagner et al., (1994) Nucleic Acids Research 22: 2114-2120, Moss et al. (1990) Nature 348:91-92; and Wagner et al., U.S. Pat. No. 5,591,601], a cytoplasmic expression vector which contains a T.sub.7 promoter connected at its 3' end to an EMC capping independent sequence into which was inserted a Lac I repressor gene and an operator sequence which provides the biding site for the Lac I repressor. The Shine-Delgarno sequence of the T.sub.7 gene was removed. A NcoI site was added to the T.sub.7 gene and the ATG codon of the T.sub.7 gene was adjusted in a manner such that translation of the T.sub.7 gene would be initiated from the ATG in the NcoI site.

US Reference Patent Number (13):
5591601

US Reference Group (13):
5591601 19970100 Wagner et al. 435/69.1

Other Reference Publication (81):
Yuyama et al. (1992) "Construction of a tRNA-embedded-ribozyme trimming plasmid," Biochem. Biophys. Res. Commun. 186:1271-1279.

CLAIMS:

7. The method of claim 6, wherein said promoter sequence further comprises a promoter sequence selected from the group consisting of tRNA, CMV, RSV, SV40, PEPCK, MT, SR.alpha., P450 family, GAL7, T.sub.7, T.sub.3, SP6, K11 and heat shock protein promoter sequences.

14. The method of claim 13, wherein said promoter sequence further comprises a promoter sequence selected from the group consisting of tRNA, CMV, RSV, SV40, PEPCK, MT, SR.alpha., P450 family, GAL7, T.sub.7, T.sub.3, SP6, K11 and heat shock protein promoter sequences.

20. The method of claim 19, wherein said promoter sequence further comprises a promoter sequence selected from the group consisting of tRNA, CMV, RSV, SV40, PEPCK, MT, SR.alpha., P450 family, GAL.sub.7, T.sub.7, T.sub.3, SP6, K11 and heat shock protein promoter sequences.